GENETICALLY MODIFIED MICROORGANISMS, PLASMID AND FERMENTATION PROCESS WITH THE PRESENCE OF FLOCCULATION REGULATED BY MEDIUM CHANGES

BACKGROUND OF THE INVENTION

Field of the invention

The present invention refers to processes and additions of flocculant genes in microorganisms for the production of industrial raw materials and process using said microorganisms.

More specifically, this application refers to genetically modified microorganisms, a process to produce said organisms and fermentation processes using said organisms. These microorganisms flocculate after the end of the fermentation process, settling more quickly in the bottom of the fermentation vessel, thus separating from the final product obtained during fermentation. This consists in a purifying process.

Description of the Prior Art

The use of microorganisms to modify and produce medical or food raw materials is known since the early history of mankind, with a basic example in the process to ferment "juices" of materials rich in sugar, such as grape, barley and other elements which, after that process, are transformed by means of anaerobic reactions into other products such as wine, beer and other compounds.

With the advent of biotechnology, many of these transformations start to be made at molecular level, and therefore can generate other kinds of raw materials such as proteins and their derivatives, strongly helping the pharmaceutical industry for the synthesis of essential medicine for the survival of human beings, such as insuline, growth hormone and others.

Some time ago, the term fermentation referred in biotechnology necessarily to anaerobic fermentation, in which microorganisms processed the raw material in the lack of air. The main products from anaerobic fermentation are alcohol, ethanol, alcoholic beverages and the bread industry.

With the advent of recombinant DNA skills, fermenters started to be used in aerobic fermentation processes. With aerobic fermentation, microorganisms are used to produce other substances such as proteins, pharmacologicals, industrial enzymes resulting from the expression of given genes naturally included in said microorganisms or introduced into these microorganisms by means of recombinant DNA skills.

An important industrial problem for fermentation processes is to separate the microorganism from the final product of interest. This step is known as purification and is made by several ways.

Purification skills are divided in two macro groups. The first skill involves the separation of the final product from larger components such as microorganisms. The second skill involves product purification after being recovered.

Concerning larger component skills, one of the ways is to use centrifuges or rotation filtration under vacuum which, by means of the centrifuge force, force the accelerated separation of substances with different densities, thus separating the final product from microorganisms.

This skill requires expensive equipments, which need huge maintenance and generate excessive loss in the process.

Another used process is decanting. As decanting is natural, it becomes very slow, such as in the case of brandy production. Furthermore, it can cause early cell disruption, launching undesirable substances into certain processes, such as the wine manufacturing process.

Cell disruption is another skill used when the product of interest is found within the cell, i. e. it has not been secreted. Disruption is made by means of chemical or mechanical agents breaking cell membranes, liberating the product into the medium.

Adsorption chromatography is another very used process but, besides being expensive, it is highly specific for each kind of product of interest.

All these processes become expensive for the delay to obtain the final product or for the high costs involved with the industrial plant required to process them.

To solve these problems, there have been various attempts to cause the more intense occurrence of the flocculation phenomenon (grouping microorganisms into lumps), so to facilitate the process to take out the unrequired material.

With the possibility of genetic modification, the phenomenon of flocculation can be used for various industrial purposes, being a part of the process to purify the product of interest. The combination of a conditional promoter with a flocculation gene optimizes the fermentation process, so to make use of the whole substract transformed into product as linked to the first purification stage. Genes intend to mean nucleotide sequences codifying peptides or proteins. Genes have a non-coding region called PROMOTER, subdividing genes into two classes: CONSTITUTIVE GENES, which are expressed apart from any stimulation and REGULATED GENES, depending on a stimulus to be expressed. On the other hand, each promoter has the own characteristics of the nucleotide sequence and position concerning the coding part, and can restrain or induce gene expression according to the components of the fermentation medium. As an example, we can mention the construction of ADH promoter adjacent to

flocculation genes, which are expressed in the lack of glucose and inhibited in the lack of said sugar.

The flocculation process is ruled by specific genes, some of which are well known in the literature, such as FLO1 from Watari (J. Watari, *Agric. Biol. Chem.*, Vol. 55, n° 6, 1991 - pages 1547 to 1552) and in patents such as Pereia (2000) in the patent PI 0001122-3 and Watari (1996) in the application US 5,585,271.

In the application US 5,585,271, Watari refers to the flocculation gene FLO1 as found in yeasts. The main focus of Watari's patent is the gene, but Watari mentions in the examples the use of the coding part of the FLO1 gene with the promoter ADH for the production of beer with yeasts *Saccharomyces cerevisiae*.

In general, this reference concerns to beer production process, but said process with flocculants presents a few disadvantages, such as low fermentation speed; formation of lumps fluctuating on the surface and making fermentation and aeration of vats used in the process become difficult; loss of cells in the fermented lather and cloggings.

Pereira (2000) has also presented a patent application (PI 0001122-3 A), in which he also uses the FLO1 gene with the ADH promoter, but applicable to the production of ethanol and wine. Said patent has three main limitations: i) it is limited to alcoholic fermentation, ii) it is limited to fermentation process (and does not cover microorganism and plasmides) and iii) it is limited to use in wild line microorganisms.

Furthermore, in Pereira's patent (2000), the FLO1 gene is used, which has incomplete and unsatisfactory flocculation standards for most industrial yeasts. FLO1L and FLO1S genes as mentioned by Watari (1996) correct this problem, since they include more sections for full flocculation.

Another patent referring to flocculation genes is Kobayashi (US 5,866,374). Said patent refers to the use of the Lg-FLO1 gene for beer production. Beer production is an alcoholic and anaerobic fermentation of no use for other substances such as pharmacologicals and industrial enzymes. Furthermore, this process does not refer to the conditional regulation of that gene with a sensible promoter at the end of the fermentation process.

That process is an attempt to enhance beer fermentation, by simply disrupting Lg-FLO1 gene or increasing its expression, by making use of the complete gene. Anyway, if the expression is enhanced or elliminated, it has only one way of operation during the whole fermentation: either it just flocculates or it never flocculates.

Oliver's patent GB 2353798 also uses flocculation genes regulated by specific promoters. But the used flocculation gene is PKC1 with promoters SRB1 and PSA1. This construction is solely used for beer production. This combination of

genes and promoters and their use for beer production are not the scope of our invention.

SUMMARY OF THE INVENTION

The object of the present invention is to obtain a genetically modified microorganism which can have flocculation genes regulated by promoters which are started or restrained, depending on characteristics of chemical composition of the medium, pH or by physical excitations.

The present application is different from the patents as mentioned above as state of the art for various reasons. First of all for the process, since all above patents described as state of the art refer to anaerobic fermentation processes, while the present application refers to aerobic fermentation processes. This difference brings unexpected effects with scientific and economical importance. The anaerobic fermentation process only allows the production of alcoholic products, such as wine, ethanol and alcoholic beverages in general. The aerobic fermentation process allows the production of other substances: e.g. proteins, pharmaceuticals, insuline, vaccines, industrial enzymes and enzymes in general. The production of these other substances results from the expression of given genes, naturally contained in said microorganisms or introduced into said microorganisms, by means of recombinant DNA skills.

The present invention also refers to anaerobic fermentation processes but, in this case, we make use of combinations of flocculation genes, promoters and microorganisms which are different from those referred to in the patents of the state of the art. These differences also bring unexpected effects with scientific and economical importance. These different combinations of flocculation genes, promoters and microorganisms have also been applied to the patent applications for microorganisms, vectors, plasmids and cassettes, which can be used for both anaerobic and aerobic fermentation processes.

The present application requires a patent not only for the process, but also for the genetically modified microorganism. A patent is also required for the vectors, plasmids and cassettes which are required to construct said genetically modified microorganism.

Watari's and Oliver's patents claim just yeasts as genetically modified microorganisms. In the present application, we have also included bacteriae, algae, protozoae, fungi and archae. All these microorganisms have very different characteristics from yeasts, e. g. they are not eucariotes, their handling is simpler and they are able to survive under high temperatures and make photosynthesis.

Pereira's patent does not claim the microorganism, vectors, plasmids and cassettes, but only claims a patent for the fermentation process.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Watari's patent mentions the use of FLO1 gene and ADH

promoter for a yeast *Saccharomyces cerevisiae* strain W204 for beer fermentation. We make use of the FLO1 gene and ADH for yeasts with different characteristics from *Saccharomyces cerevisiae*, such as *Pichia pastoris*, *Hansenula polymorpha*, *Saccharomyces fragilis*, *Saccharomyces ellipsoideus*, *Saccharomyces calsbergensis*, *Candida utilis*, *Candida lipolytica* or *Kluyveromyces lactis*. These other yeasts are extremely more productive than *Saccharomyces cerevisiae* or have different fermentation effects.

Pichia pastoris and *Hansenula polymorpha* have very large productiveness for the production of proteins coded by genes located in given regions. They are used as highly efficient expression vectors, which are much more efficient than *Saccharomyces cerevisiae*.

E. g. *Saccharomyces ellipsoideus* is used to ferment wines. E. g. *Saccharomyces calsbergensis* is used for "lager" beer production, while *Saccharomyces cerevisiae* is used for "ale" beer production. *Candida utilis*, *Candida lipolytica* and *Kluyveromyces lactis* are used to ferment ethanol.

Among valid strains, the following are included:

956. X2180-1A, ATCC 26786 a SUC2 mal gal2 CUP1 R. K. Mortimer

957. X2180-1B, ATCC 26787 alpha SUC2 mal gal2 CUP1 R. K. Mortimer

857. 158 a *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

858. 159 alpha *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

713. KIL-k2 From draught beer) Antonie van Leeuwenhoek, 1978, 44, 59 M. Richards

738. KIL-k2 (brewery contaminant) Antonie van Leeuwenhoek, 1978, 44, 59 A. P. Maule

761. KIL-k3 From palm wine), CBS 7903 *J. Ferm. Technol.*, 1985, 63, 421-429 N. Okafor

1001. KIL-k2 (brewing yeast) Antonie van Leeuwenhoek, 1978, 44, 59

1561. A8209B his4-864 KIL-k1 G. Fink via T. Young

958. X2928-3D-1A a ade1 gal1 leu1 his2 ura3 trp1 met14 R. K. Mortimer

959. X2928-3D-1C alpha ade1 gal1 leu1 his2 ura3 trp1 met14 R. K. Mortimer

1786. STX 147-4C alpha ade1 his7 tyr1 gal1 cly8 ade5 aro2 met13 lys5 trp5 cyh2 arg4 lys1 ura4 gal2 ade2 rad56 L. Johnston

1620. STX77-6C alpha gal1 his4 trp1 hom3 ura3 CUP1 ilv3 ade3 rad52 rna1 L. Johnston

1618. X4119-19C a his7 tyr1 cdc9 trp4 aro1B hom2 rad2 thr1 lys11 gal2 ade2 L. Johnston

1661. X4120-19D alpha lys2 leu2 pet14 rad(?) rna3 ade8 aro1D met10 ade5 leu1 CUP1 L. Johnston

1619. STX66-4A a rad18 lys4 trp1 prt3 CUP1 gal2 ade2 met2 pha2 L. Johnston

1617. K396-22B alpha spo11 ura3 ade1 his1 leu2 lys7 met3 trp5 L. Johnston

1614. K381-9D alpha spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1 L. Johnston

1613. K398-4D a spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1 L. Johnston

1611. K382-23A a spo11 ura3 can1 cyh2 ade2 his7 hom3 L. Johnston

1612. K382-19D alpha spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1 L. Johnston

1616. K393-35C alpha spo11 ura3 his2 leu1 lys1 met4 pet8 L. Johnston

1615. K399-7D a spo11 ura3 his2 leu1 lys1 met4 pet8 L. Johnston

1383. DBY 747, ATCC 44774 a leu2-3 leu2-112 his3-DELTA1 trp4-289 ura3-52 Gene, 1979, 8, 17-24. J. F. Makins

1392. MC16 alpha leu2-3 his4-712FS) SUF2 ade2-1 lys2-1 Nature, 1981, 275, 104. J. F. Makins

1445. LL20 alpha his3-11 his3-15 leu2-3 leu2-112 *J. Bacteriol.*, 1979, 140, 73-82 A. Coddington

1527. MD40/4C alpha leu2-3 leu2-112 his3-11 his3-15 ura2 trp1 CAN s B. Bowen

1528. AH22 a leu2-3 leu2-112 his4 canR B. Bowen

1627. D13-1A (YNN6) a trp1 his3-532 gal2 L. Johnston

1771. cdc9-1 (L89-6C) a cdc9-1 leu2 ade1 ade2 ura1 lys2 L. Johnston

804. D160 a ura3 his1 arg6 trp2 ade1 J. F. T. Spencer

805. A364A a ade1 ade2 ura1 his7 lys2 tyr1 gal1 *J. Mol. Biol.*, 1976, 105, 427-443 J. F. T. Spencer

806. x112 alpha ade8-2 trp5-2 lys2-1 ura1-1 J. F. T. Spencer

808. GRH1 a trp1 ade1 his7 ura1 gal1 G. Stewart

1395. S1896D a met7 trp1 leu1 ade1 gal1 gal2 pet R. K. Mortimer

1396. F33 alpha met7 gal2 pet R. K. Mortimer

1623. X2181-1B a gal1 his2 trp1 ade1

1626. CG379 ade+ alpha his7-2 leu2-3 leu2-112 trp1-289 ura3-52 (ade5 rev)

1631. g440-7C alpha ade4 trp1 L. Johnston

1632. D273-11A alpha ade1 his1 trp2 L. Johnston

1662. alpha arg met L. Johnston

1663. a arg met L. Johnston

1664. alpha/alpha arg 2μ + L. Johnston

1719. A364A rho- a ade1 ade2 ura1 his7 lys2 tyr1 gal1 rho- L. Johnston

1720. B635 a cyc1-115 his1-1 lys2-1 trp2 L. Johnston

1772. L126-R9 a leu2 hom3-10 his1 L. Johnston

1790. a/a arg his 2μ + L. Johnston

1812. L126-2B a leu2 hom3-10 his1 L. Johnston

1821. S13 a his4 ura1 trp5 gal2 L. Johnston

1822. S49 a his4 ura1 trp5 gal2 ade6 L. Johnston

1961. GRF18 alpha leu2-3 leu2-212 his3-11 his3-15 canR G. Fink via D. MacKenzie

1356. A 137 alpha pho80-2 *J. Bacteriol.*, 1973, 113, 727-738 A. Coddington

1357. A 138 a pho80-2 *J. Bacteriol.*, 1973, 113, 727-738 A. Coddington

828. a ade1 J. F. T. Spencer
829. alpha ade1 J. F. T. Spencer
1577. a ade1 leu1 B. Pearson
1652. a ade1 leu2 B. Pearson
830. a ade2 J. F. T. Spencer
802. alpha ade2 (lys) J. F. T. Spencer
832. a ade3 J. F. T. Spencer
833. alpha ade3 (ura) J. F. T. Spencer
834. a ade4 J. F. T. Spencer
835. alpha ade4 (ura) J. F. T. Spencer
836. a ade5 J. F. T. Spencer
837. alpha ade5 (ura) J. F. T. Spencer
838. a ade6 J. F. T. Spencer
839. alpha ade6 (trp) J. F. T. Spencer
840. a ade7 J. F. T. Spencer
841. alpha ade7 J. F. T. Spencer
842. a ade8 J. F. T. Spencer
843. alpha ade8 (lys trp) J. F. T. Spencer
1654. cdc3-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc3-1 L. Johnston
1642. cdc4-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc4-1 L. Johnston
1643. cdc5-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc5-1 L. Johnston
1723. cdc6-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc6-1 L. Johnston
1729. cdc7-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc7-1 L. Johnston
1730. cdc8-141 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc8-141 L. Johnston
1667. cdc8-198 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc8-198 L. Johnston
1771. cdc9-1 (L89-6C) a cdc9-1 leu2 ade1 ade2 ura1 lys2 L. Johnston
1788. cdc9-1 rev1 cdc9-1 rev1 L. Johnston
1672. cdc9-12 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-12 L. Johnston
1673. cdc9-13 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-13 L. Johnston
1791. cdc9-3 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-3 2μ+ L. Johnston
1731. cdc9-4 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-4 L. Johnston
1732. cdc9-6 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-6 L. Johnston
1807. cdc9-7 (L82-2B) a cdc9-7 trp1 lys2 his7 L. Johnston
1808. cdc9-7 (L94-4D) a cdc9-7 trp1 ura3 L. Johnston
1670. cdc9-7 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-7 L. Johnston
1633. cdc9-7 rho- cdc9-7 rho- L. Johnston
1671. cdc9-8 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc9-8 L. Johnston
1674. cdc10-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc10-1 L. Johnston

1655. cdc11-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc11-1 L. Johnston
1733. cdc12-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc12-1 L. Johnston
1734. cdc13-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc13-1 L. Johnston
1735. cdc14-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc14-1 L. Johnston
1736. cdc18-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc18-1 L. Johnston
1737. cdc19-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc19-1 L. Johnston
1738. cdc26-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc26-1 L. Johnston
1665. cdc28-4 L31-7a a cdc28-4 tyr1 L. Johnston
1675. cdc30-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc30-1 L. Johnston
1676. cdc31-1 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc31-1 L. Johnston
1722. cdc36-16 SR661-2 a cdc36-16 trp1-1 ura1 L. Johnston
1666. cdc37-1 SR672-1 a cdc37-1 ura1 cyh2 L. Johnston
1641. cdc39-1 SR665-1 alpha cdc39-1 met2 tyr1 cyh2 L. Johnston
1677. cdc41 a ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc41 L. Johnston
1753. cdc6 (MH18) cdc6/cdc6 gal1/gal1 ade1/ade1 +/ade2 his1/his7 trp2/+ +/ura1 +/lys2 +/tyr1 2μ+ L. Johnston
1754. cdc13 (MH20) cdc13/cdc13 gal1/gal1 ade1/ade1 +/ade2 his1/his7 trp2/+ +/ura1 +/lys2 +/tyr1 2μ+ L. Johnston
1752. cdc15-1 (MH15) cdc15-1/cdc15-1 gal1/gal1 ade1/ade1 +/ade2 his1/his7 trp2/+ +/ura1 +/lys2 +/tyr1 2μ+ L. Johnston
1755. cdc17 (MH21) cdc17/cdc17 gal1/gal1 ade1/ade1 +/ade2 his1/his7 trp2/+ +/ura1 +/lys2 +/tyr1 2μ+ L. Johnston
1756. cdc21 (MH21) cdc21/cdc21 gal1/gal1 ade1/ade1 +/ade2 his1/his7 trp2/+ +/ura1 +/lys2 +/tyr1 2μ+ L. Johnston
1718. JC2 (L31-9a [a]/L31-2c [alpha]) cdc9/cdc9 cdc28/cdc28 ade/+ tyr1/tyr/1 canR/+ +/his1 L. Johnston
1797. cdc36(MH30) alpha/a gal1/gal1 ade1/+ his1/+ trp2/+ +/trp1 +/ura1
961. 2C-4 alpha arg4-2/+ arg4-17/+ CUP1/+ thr1/+; rec5 leu1 trp5 trp1 his5 ade2 *Radiation Res.*, 1972, 49, 133 & 148 R. K. Mortimer
960. 2C-8 alpha arg4-2/+ arg4-17/+ CUP1/+ thr1/+; rec4 leu1 trp5 trp1 his5 ade2 *Radiation Res.*, 1972, 49, 133 & 148 R. K. Mortimer
1823. dbf1 (L123-8A) alpha trp1 ura3 dbf1 L. Johnston
1824. dbf2 (L119-7D) alpha trp? ura3 ade1 dbf2 L. Johnston
1750. dbf2-3 (D128) alpha ade1 his1 trp2 dbf2-3 L. Johnston
1825. dbf3 (L124-11D) a ura3 dbf3 L. Johnston
1751. dbf3-1 (D128) alpha ade1 his1 trp2 dbf3-1 L. Johnston
1747. dbf8-1 (D128) alpha ade1 his1 trp2 dbf8-1 L. Johnston
1748. dbf9-1 (D128) alpha ade1 his1 trp2 dbf9-1 L. Johnston

1813. dbf10-1 (D141) alpha ade1 his1 trp2 dbf10-1 L. Johnston
1814. dbf11-1 (D132) alpha ade1 his1 trp2 dbf11-1 L. Johnston
1815. dbf13-1 (D101) alpha ade1 his1 trp2 dbf13-1 L. Johnston
1816. dbf14-1 (D22) alpha ade1 his1 trp2 dbf14-1 L. Johnston
1817. dbf14-2 (D25) alpha ade1 his1 trp2 dbf14-2 L. Johnston
1818. dbf14-3 (D44) alpha ade1 his1 trp2 dbf14-3 L. Johnston
1819. dbf15-1 (D22) alpha ade1 his1 trp2 dbf15-1 L. Johnston
1820. dbf20-1 (D175) alpha ade1 his1 trp2 dbf20-1 L. Johnston
1794. MH25 alpha/a dbf2/dbf2 gal1-D5/gal1-A ade1/+ his1/his1 trp2/trp2 +/ura1 +/tyr1 +/ade2 +/his L. Johnston
1795. MH26 alpha/a dbf3/dbf3 gal1-D5/gal1-a ade1/ade1 his1/+ trp2/+ +/lys2 +/ura1 +/tyr1 +/ade2 L. Johnston
1796. MH27 alpha/a dbf4/dbf4 gal1-D5/gal1-A ade1/ade1 his1/his1 trp2/+ +/lys2 +/tyr1 +/ade2 +/his7 L. Johnston
1621. g716-5a ho a can1 hom3-10 his1-7 L. Johnston
1622. 309 alpha ade2-R8 metX can1-11 L. Johnston
1717. L39-8C alpha trp1 or trp2 lys2 canR L. Johnston
1628. 320 a rme ade2 ura3 leu1 can1-11 cyh2-21 L. Johnston
1716. alpha131-20 alpha ade2-R8 cyh2 can1 leu1 ura3 L. Johnston
807. x 464-20C alpha trp1 ade1 his2 leu1 gal1 J. F. T. Spencer
859. 168 a ade1 gal1 lys2 tyr1 his7 ura1 ade2 *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart
1789. L58.3b gal1 ade1 or ade2 L. Johnston
1638. L57-15b gal1 his7 lys2 tyr1 L. Johnston
1625. Z65 a/alpha gal1-1/gal1-4 lys2-1/lys2-2 tyr1-1/tyr1-2 his7-2/his7-1 ade1/+ +/ade2 +/ura L. Johnston
1757. M1-2B (YNN 27) alpha trp1 ura3-52 gal2 L. Johnston
865. 205 alpha gal7 lys2 tyr1 his4 leu2 thr4 MAL2 trp1 ade6 arg4 ura4 suc- *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart
866. 206 a gal7 lys2 tyr1 his4 MAL2 trp1 ade6 arg4 suc- *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart
1624. 108-3A a gal80 ade6 thr4 trp1 his3 rho- L. Johnston
1636. L53-14C a gal80 gal1-A tyr1 lys2 his7 ade1(or ade2) ura1 L. Johnston
1635. L52-36 alpha gal80 gal1-D5 ade1 his1 (or his8) trp1(or trp8) L. Johnston
1787. 106-3D alpha gal80 ura1 his1 L. Johnston
1634. MH10 alpha/a gal80/gal80 gal1-D5/gal1-A trp2/+ +/ura1 +/tyr1 +/lys2 +/his2 +/ade1 or 2 L. Johnston
867. 207 a ade1 gal1 ura3 his2 trp5 leu1 lys7 met2 MAL3 SUC2 *Can. J. Microbiol.*, 1977,

23, 441 G. Stewart

864. 194 a ade1 trp5 MAL6 suc- *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

862. 191 a ade2 MAL3 SUC3 MEL1 MGL2 MGL3 *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

861. 190 a his4 leu2 MAL2 suc- *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

863. 192 a trp1 ura3 MAL4 MEL1 MGL3 suc- gal3 gal4 *Can. J. Microbiol.*, 1977, 23, 441 G. Stewart

2252. a/alpha trp1/+ his2/+ ade1/+ STA2/STA2 *Biochem. J.*, 1988, 249, 163 I. Evans

860. 169 alpha ilv2 his FLO1FLO4) G. Stewart

868. 209 a ilv2 FLO1FLO4) G. Stewart

869. 209 alpha FLO1FLO4) G. Stewart

870. 210 a ade1 gal1 trp1 ura3 his2 leu1 met14 FLO1FLO4) G. Stewart

1391. sigma 1278b wild type (parent) *J. Bacteriol.*, 1970, 103, 770 R. Robbins

1390. 2512C a gap1 *J. Bacteriol.*, 1970, 103, 770 R. Robbins

1454. MP1, ATCC 42131 a/alpha ade2/+ his8/+ trp5-12/trp5-21 R. Fahrig

916. JCK5-5A alpha his4-A15 ade2-1 can(R) kar1-1 J. Conde

917. ABq 21 alpha his4-A15 ade2-1 can(R) kar1-2 nys(R) J. Conde

2266. BC3 leu2-3.112 trp1.1 ura3-52 pgk::TRP1 *Nucl. Acids Res.*, 1988, 16, 1333-1348 P. Piper

1639. a rad1 rad18 1799. CM31/1d alpha rad1 leu his ade lys L. Johnston

1800. CM26/4c rad4-3 his leu L. Johnston

1763. CM4/1d alpha rad5 ura L. Johnston

1764. CM5/1b alpha rad7 leu L. Johnston

1805. CM21/9a a rad9 ade arg leu lys L. Johnston

1806. CM30/2C alpha rad11 ade arg his leu L. Johnston

1801. CM1/8a alpha rad18 ade2 leu2 his4 L. Johnston

1640. g739-2a a rad50-1 can1 his1 ade2 (or adeX) L. Johnston

1721. g739-2d alpha rad50-1 hom3-10 his1 trp2 L. Johnston

1802. CM1/1C alpha rad51 lys2 leu2 his4 L. Johnston

1803. CM8/1a a rad54 ura his leu L. Johnston

1804. CM9/1a a rad55 leu his L. Johnston

1749. g725-12a alpha rad57-1 gal1-D5 hom3-10 his1-7 L. Johnston

1630. SK1 (L57.15b/L58.3b) gal1 het3 his7/+ lys2/+ tyr1/+ gal1-A/gal1-D5 +/ade1 (or ade2) homothallic L. Johnston

1637. g761-10A [alpha]/g763-5c [a] rad51-3/rad51-3 gal1-A/gal1-5 his1-1/his1-7 +/his6 +/his7 tyr?/+ lys?/+ trp?/+ +/hom3-10 +/spo13-1 +/lys? +/tyr1 +/ura1 +/ade2

1792. g650-4a [alpha]/g650-12a[a] rad52-1/rad52-1 CAN(s)/can(R) +/hom3-10 +/his1-7 +/trp ade4/+ ho/ho

1745. ma3-3 (D43) alpha ade1 his1 trp2 rna3-3 L. Johnston
1746. ma3-4 (D167) alpha ade1 his1 trp2 ma3-4 L. Johnston
1758. ts96 alpha ade1 his1 trp2 rna11-2 dds1-1 L. Johnston
1614. K381-9D alpha spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1 L. Johnston
1612. K382-19D alpha spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1 L. Johnston
1611. K382-23A a spo11 ura3 can1 cyh2 ade2 his7 hom3 L. Johnston
1616. K393-35C alpha spo11 ura3 his2 leu1 lys1 met4 pet8 L. Johnston
1617. K396-22B alpha spo11 ura3 ade1 his1 leu2 lys7 met3 trp5 L. Johnston
1613. K398-4D a spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1 L. Johnston
1615. K399-7D a spo11 ura3 his2 leu1 lys1 met4 pet8 L. Johnston
227. Strain K, Manchester brewery strain, 1:5:4:2:1.
228. Strain R, Sheffield brewery strain, 5:1:1:3:5.
229. Strain T, London brewery strain, 5:1:1:4:5.
230. Strain U, Birmingham brewery strain, 5:1:1:4:5.
231. Strain V, Burton-on-Trent brewery strain, 1:5:5:3:1.
232. Strain S, American Yeast Foam, ATCC 60782, 1:1:3:5:1.
205. Hybrid 1 (NCYC 227 x NCYC 228)
206. Hybrid 2 (NCYC 227 x NCYC 229)
207. Hybrid 3 (NCYC 227 x NCYC 230)
208. Hybrid 4 (NCYC 227 x NCYC 230)
209. Hybrid 5 (NCYC 227 x NCYC 231)
210. Hybrid 6 (NCYC 227 x NCYC 231)
211. Hybrid 7 (NCYC 230 x NCYC 231)
212. Hybrid 15 (NCYC 227 x NCYC 232)
213. Hybrid 18 (NCYC 220 x NCYC 232)
214. Hybrid 24 (NCYC 222 x NCYC 221)
215. Hybrid 30 (NCYC 223 x NCYC 221)
216. Hybrid 38 (NCYC 224 x NCYC 226)
217. Hybrid 39 (NCYC 225 x NCYC 226)
218. Hybrid 48 (NCYC 226 x A162/1 ex NCYC 216)
219. Hybrid 64 (NCYC 227 x A162/3 ex NCYC 216)
220. Single spore isolate A2/3 strain from NCYC 212
221. Single spore isolate A38/3 strain from NCYC 213
222. Single spore isolate A48/1 strain from NCYC 213
223. Single spore isolate A85/1 strain from NCYC 214
224. Single spore isolate A101/1 strain from NCYC 214
225. Single spore isolate A101/2 strain from NCYC 214
226. Single spore isolate A104/1 strain from NCYC 214

646. x901-35C strain; alpha hom2 aro1A trp5 leu1 ade6 lys1 his6 ura1 arg4-1 thr1
647. x901-26A strain; alpha hom2 aro1A trp5 leu1 ade6 his6 ura1 arg4-2 thr1
648. x1069-1A strain; a ade1 his4 leu2 thr4 met2 trp5 ura1
650. DV 147 strain; alpha ade2, readily reverts to wild type
651. 4B strain; alpha his4 leu3 lys10(?) ade6 ade2 met(?),
652. S400D strain; a ilv1; has other unlisted requirements
653. S288C-27 strain; alpha ilv1, has other unlisted requirements
654. S2583D strain; alpha ilv2, has other unlisted requirements
655. S2582B strain; alpha ilv2, has other unlisted requirements
656. JB19 strain; alpha leu1 ade2
657. JB143 strain; alpha leu2 ade2
658. JA36 strain; a leu3 ade2 lys10
659. x 764 diploid hybrid strain; segregates for markers trp5 leu1 ade6 ura3 hom3 his6 lys1 arg4 mal1
660. x 373 tetraploid hybrid strain
661. x 362 hexaploid hybrid strain
663. xJ151 hybrid diploid strain; ATCC 60732; segregates for markers thr1 lys1 ura3 aro1A hom2 trp4 ade8; homozygous for ade2
664. xJ107 hybrid diploid strain; segregates for markers leu1 ura3 lys7 gal7 his8 ser1 ade2
264. S. Jackson Farmer's diploid strain 18, C53-8d x C24-13b) 1959
402. A. A. Eddy F28c strain, single spore isolate from NCYC 264) 1953
593. W. F. F. Oppenoorth (R7, O. Winge's C.L.303-9 hybrid strain) 1959
594. W. F. F. Oppenoorth (K83 S 58 hybrid strain) 1959
666. J. W. Millbank (respiratory deficient mutant derived from ale yeast NCYC 239) 1963
673. H. Laser (petite colony mutant by x-irradiation of baker's yeast) 1963
505. CBS (1957). CBS 1171, ATCC 18824. Type strain for *Saccharomyces cerevisiae*. From brewing yeast. 5:1:5:5:1
70. A. C. Chapman (1933). *Saccharomyces anamensis*. NCTC 3864.
72. Schmitt (1924). *Saccharomyces brasiliensis*. 98 Carlsberg strain, NCTC 1808.
74. ATCC (1945). *Saccharomyces carlsbergensis*. ATCC 9080, ATCC 24904, CBS 2354.
76. A. C. Chapman (1933). *Saccharomyces cartilaginosus*. NCTC 3865.
77. A. Harden (1921). Baker's yeast strain. Requires thiamin, pantothenate and biotin (*Arch. Biochem.*, 1947, 14, 369. *J. Gen. Microbiol.*, 1983, 128, 2615-2620).
78. A. C. Chapman (1925). NCTC 2160
79. ATCC (1942). ATCC 7754, CBS 1368, NRRL Y-977, IFO 1346. Fleischmann baker's strain. Assay of biotin.
80. H. B. Hutchinson (1930). GB 354, NCTC 5922.

81. ATCC (1942). ATCC 7752, CBS 1320, NRRL Y-973, IFO 1234. Gebrüder Mayer Strain.

82. A. Klocker (1920). NCTC 466.

83. A. Harden (1920). Carlsberg Laboratory strain 21, NCTC 381.

84. H. J. Bunker (1945). NCTC 7043.

85. A. J. Kluyver (1939). NCTC 5916.

86. ATCC (1942). ATCC 7753, CBS 1321, NCTC 6421.

87. ATCC (1947). ATCC 9763, NRRL Y-567, CBS 2978, NCTC 10716 and NCTC 7239.

89. ATCC (1946). ATCC 7921. From Fleischmann yeast cake. (*J. Phys. Chem.*, 1928, 32, 1094).

90. A. Castellani (1928). NCTC 2779. Distiller's yeast.

91. A. Guilliermond (1925). *Saccharomyces chevalieri*. CBS 400, ATCC 9804, NCTC 2054. Type strain for *Saccharomyces chevalieri*. From wine.

92. A. C. Chapman (1933). *Saccharomyces delbrueckii*. NCTC 3964.

93. A. C. Chapman (1925). *Saccharomyces cerevisiae var. ellipsoideus*. NCTC 2161. Wine yeast.

94. A. Klocker (1920). *Saccharomyces ellipsoideus*. NCTC 467, NRRL Y-129, ATCC 2338.

95. A. C. Chapman (1933). *Saccharomyces ellipsoideus var. cratericus*. NCTC 3866.

96. M. B. Church (1922). *Saccharomyces cerevisiae var. ellipsoideus*. NCTC 1344. For the production of vinegar from apple juice.

97. H. B. Hutchinson (1945). *Saccharomyces ellipsoideus*. Michigan 48 strain. ATCC 10824, NCTC 7040.

99. J. L. Baker (1930). *Saccharomyces festinans*. From infected ale (*J. Inst. Brew.*, 1929, 35, 466).

104. M. Kir (1934). Hungarian Wine Yeast.

107. A. J. Kluyver (1939). *Saccharomyces intermedius*.

108. T. Castelli (1939). *Saccharomyces italicus*. From Chianti grape must.

109. Carlsberg Laboratory (1924). *Saccharomyces lactis*.

110. A.Guilliermond (1925). *Saccharomyces lindneri*. CBS 403. From West African ginger beer.

113. A.Guilliermond (1925). *Saccharomyces vini*.

118. Gray, McGill University (1932). *Saccharomyces sake*.

121. A. C. Chapman (1921). *Saccharomyces thermantitonum*.

122. B.von Euler (1921). *Saccharomyces thermantitonum*. (*Biochem. Z.*, 1919, 97, 156).

124. A. Klocker (1920). NRRL Y-2434 *Saccharomyces turbidans*. (*J. Inst. Brew.*, 1950, 56, 192).

125. A. Heinemann (1933). *Saccharomyces ellipsoideus*. (*Exp. Cell. Res.*, 1958, 15, 214).

126. A. Klocker (1920). *Saccharomyces validus.*

167. B. W. Hammer (1922). *Torula cremoris.* From fermented cream.

176. A. J. Kluyver (1934). *Zygosaccharomyces priorianus.*

177. Anheuser-Busch Inc., U. S. A. (1927). Californian wine yeast. (*J. Gen. Microbiol.*, 1982, 128, 2615-2620).

182. A. C. Chapman (1933). Fernbach 38 strain.

183. A. C. Chapman (1933). Fernbach 40 strain.

186. Ö. Winge via British Fermentation Products Ltd. (1942). Hybrid K471.

187. A. C. Chapman (1921). Kefir Yeast.

190. A. C. Chapman (1931). *Saccharomyces logos.* ATCC 60731, NCTC 3341. Killer character K1 (Antonie van Leeuwenhoek, 1978, 44, 59-77).

192. G. Johnson (1936). Melbourne No. 1 strain.

196. A. C. Chapman (1933). Yeast Race V.

197. Mehta (1925). Yeast Race II.

198. Institut für Garungsgewerbe (1925). Yeast Race XII.

199. A. C. Chapman (1921). Saaz Yeast. NCTC 906, ATCC 2704, NRRL Y-239. From Bohemian brewery.

200. A. C. Thaysen (1920). Stemberg 675 strain. For production of glycerol.

201. R. M. Nattrass (1943). 61 strain.

202. Carlsberg Laboratory (1924). Wine yeast, Johannesburg II Wortmann 76 strain.

232. R. S. W. Thorne (1951).

S, American Yeast Foam. 1:1:3:5:1 ATCC 60782. Killer character K1 (Antonie van Leeuwenhoek, 1978, 44, 59-77; *J. Ferm. Technol.*, 1985, 63, 421-429).

235. B. M. Brown (1951). Whitbread strain. ATCC 60733. Killer character K1 (Antonie van Leeuwenhoek, 1978, 44, 59-77). 5:1:4:4:1.

244. CMI (1951). *Saccharomyces intermedius.* IMI 46336.

252. S. Jackson (1961). *Saccharomyces ellipsoideus.* Strain 6.

291. S. Jackson via C. C. Lindegren (1951). Mrak 93 strain.

311. S. Jackson (1951). Benskin's brewery strain 65.

325. A. E. Wiles (1951). T43 Yorkshire type yeast. (*J. Inst. Brew.*, 1950, 56, 183).

341. A. E. Wiles (1951). From draught beer. 1:1:5:5:1

343. A. E. Wiles (1951). From draught beer. 1:1:5:5:1 (*J. Inst. Brew.*, 1950, 56, 183).

344. A. E. Wiles (1951). *Saccharomyces cerevisiae var. turbidans.* From draught beer. (J. Inst. Brew., 1950, 56, 183).

345. A. E. Wiles (1951). *Saccharomyces cerevisiae var. turbidans.* From draught beer. (*J. Inst. Brew.*, 1950, 56, 183).

346. A. E. Wiles (1951). *Saccharomyces cerevisiae var. turbidans.* From draught beer. (*J. Inst. Brew.*, 1950, 56, 183).

356. C. H. Ridge (1953). Mead yeast.

357. T. Gray (1951). Avize-Cramant mead yeast.

358. T. Gray (1951). Plum mead yeast.

360. D. R. Jackson (1952). Seagram & Sons.

361. R. B. Gilliland (1952). *Saccharomyces diastaticus*. CBS 1782, NRRL Y-2416, ATCC 13007, IFO 1046. Type strain for *Saccharomyces diastaticus*. From brewer's wort.

365. F. W. Beech (1952). *Saccharomyces cerevisiae var. ellipsoideus*. From apple juice.

374. L. Hemmons (1953). *Saccharomyces oviformis*. From hazy ale.

394. A. A. Eddy (1954). *Saccharomyces chevalieri*.

406. R. B. Gilliland (1954). *Saccharomyces steineri*.

410. R. B. Gilliland (1954). *Saccharomyces fructuum*.

429. L. J. Wickerham (1955). Flor yeast. NRRL Y-2036.

430. L. J. Wickerham (1955). Riesling wine yeast. NRRL Y-2037.

431. L. J. Wickerham (1955). NRRL Y-132, ATCC 2345, ATCC 44732, NCYC 73.

447. J. S. Hough (1955). *Saccharomyces diastaticus*. From draught beer.

463. H. Aebi (1955). *Saccharomyces cerevisiae var. ellipsoideus*. Riesling wine yeast, Herrliberg strain.

478. IFO (1956). Koykai 6 strain Sake yeast.

479. IFO (1956). Koykai 7 strain Sake yeast.

480. IFO (1956). R28. Awamori yeast.

481. IFO (1956). K71. Awamori yeast.

482. R. Barrington-Brock (1956). *Saccharomyces oviformis*. Champagne yeast, Moussec strain.

487. J. Lodder (1957). Single cell isolate from baking yeast Requires inositol, pantothenate, biotin and thiamin.

488. J. Lodder (1957). Single cell isolate from baking yeast. Requires inositol, pantothenate and biotin; used in copper resistance studies (*Trans. Brit. Mycol. Soc.*, 1981, 77, 27).

489. J. Lodder (1957). Single cell isolate from baking yeast. Requires inositol, pantothenate and biotin.

490. J. Lodder (1957). Single cell isolate from baking yeast.

491. J. Lodder (1957). Single cell isolate from baking yeast. Requires inositol, pantothenate, biotin and thiamin.

506. CBS (1957). *Saccharomyces cerevisiae var. ellipsoideus*. CBS 1395, NRRL Y-1529. Type strain for *Saccharomyces ellipsoideus*.

510. CBS (1957). *Saccharomyces validus*. CBS 1541. Type strain for *Saccharomyces validus*.

525. R. Ryden (1958). Baking yeast.

592. W. F. F. Oppenoorth (1959). *Saccharomyces chevalieri*. W332. Used as DNA donor. (Brauwissenschaft, 1959, 12, 103).

609. M. P. Scarr (1960). *Saccharomyces fructuum*. From West Indian molasses.

618. A. A. Eddy (1962). *Saccharomyces cartilis*.

619. CBS (1962). CBS 2184. Jerez sherry yeast from Feduchy.

620. CBS (1962). *Saccharomyces fructuum*. CBS 3012. Jerez sherry yeast from Feduchy.

621. CBS (1962). *Saccharomyces fructuum*. CBS 3013. Jerez sherry yeast from Feduchy.

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626. CBS (1962). *Saccharomyces oviformis*. CBS 429, NRRL Y-1356, IFO 0262. Type strain for *Saccharomyces oviformis*.

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671. F. W. Beech (1964). *Saccharomyces capensis*. AWRI 81. Sherry yeast.

672. F. W. Beech (1964). VY22. Sherry yeast.

673. H. Laser (1963). Petite colony mutant by X-ray irradiation of baking yeast.

684. H. J. Bunker (1965). *Saccharomyces ellipsoideus*. Steinberg wine yeast.

694. F. R. Elliot (1966). Hybrid baking yeast. Distillers strain DCL 2984 from Distillers Co. Ltd.

695. ATCC (1966). ATCC 9896. Fleischmann 139 strain.

700. CBS (1966). *Saccharomyces steineri*. CBS 423, NRRL Y-1536, ATCC 2367, IFO 0253. Type strain for *Saccharomyces steineri*. From wine.

703. S. C. Hall (1967). From draught beer. Non-fining yeast.

713. M. Richards (1967). *Saccharomyces diastaticus*. ATCC 36902. From draught beer.

716. E. Minárik (1968). Thermophilic strain.

726. R. B. Gilliland (1970). 1430 Gilliland Class IV. (*Bull. Anc. Etud. Brass. de Louvain*, 1970, p59).

727. R. B. Gilliland (1970). 1511 Gilliland Class I. (*Bull. Anc. Etud. Brass. de Louvain*, 1970, p59).

738. A. P. Maule (1972). ATCC 36900. From continuous fermentation plant.

739. L. Penasse (1972). From air. High sterol content.

748. B. E. Kirsop (1972). From "bees wine" culture.

753. B. H. Kirsop (1973). From NCYC 240. Unable to ferment maltotriose.

754. B. H. Kirsop (1973). From NCYC 240. Able to ferment maltotriose.

755. G. G. Stewart (1973). Labatt's A. (*Proc. Amer. Soc. Brew. Chem.*, 1972, 3, 1-176,

118).

756. G. G. Stewart (1973). Labatt's B. (*Proc. Amer. Soc. Brew. Chem.*, 1972, 3, 1-176, 118).

757. R. A. Coutts (1972). Used for infection of yeast protoplasts with tobacco mosaic virus (*Nature*, 1972, 240, 466).

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767. I. Campbell (1974). *Saccharomyces prostoserdovii*. as CBS 5155. T

812. J. M. Haslam (1974). KD 115, a ole1.

816. A. V. Hood (1974). AWRI 729, CECT 11133. Known as Epernay yeast.

817. J. A. Barnett (1975). as CBS 1172, ATCC 6037.

826. ATCC (1976). ATCC 26109, X-2180.

853. ATCC (1976). ATCC 2601, CBS 679, NRRL Y-53.

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914. R. B. Gilliland (1978). *Saccharomyces diastaticus*. C608.

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922. B. H. Kirsop (1979). X18. Very flocculent killer strain from batch fermentation.

923. B. H. Kirsop (1979). X19. Non-flocculent killer strain from batch fermentation.

933. J. Atputharajah (1979). *Saccharomyces chevalieri*. CRI 30. From toddy.

934. J. Atputharajah (1979). *Saccharomyces chevalieri*. CR1 Y11. From toddy.

935. J. Atputharajah (1979). *Saccharomyces chevalieri*. CR1 170. From toddy.

990. Chivas Bros Ltd (1981). *Saccharomyces diastaticus*.

991. Chivas Bros Ltd (1981). *Saccharomyces diastaticus*.

994. Chivas Bros Ltd (1981). *Saccharomyces diastaticus*. From bottled red wine.

995. S. I. Lesaffre et Cie (1981). Hybrid baking yeast. (U. S. Patent 4,396,632).

996. S. I. Lesaffre et Cie (1981). Hybrid baking yeast. (U. S. Patent 4,396,632).

999. All-Union Collection of Non-pathogenic Microorganisms (1981). Strain 383, Fleischmann Yeast Race xii, No. 46.

1370. B. E. Kirsop (1981). From wine packing cellar. Sporulates abundantly.

1379. New Zealand brewery (1981). Wild yeast.

1380. New Zealand brewery (1981). Wild yeast.

1406. S. Hara (1981). WL-7, IAM 4098 (*Agric. Biol. Chem.*, 1981, 45, 1327-1324. *Am. J. Enol. Vitic.*, 1980, 31, 28-37).

1407. S. Hara (1981). KL-88 Killer, sake strain. (*Agric. Biol. Chem.*, 1981, 45, 1327-1324. *Am. J. Enol. Vitic.*, 1980, 31, 28-37).

1408. S. Hara (1981). OC-2, IAM 4274. Mesophilic wine yeast. (*Agric. Biol. Chem.*, 1981,

45, 1327-1324. *Am. J. Enol. Vitic.*, 1980, 31, 28-37).

1409. S. Hara (1981). 2HYL-2. Hybrid NCYC 1406 x 1407. (*Agric. Biol. Chem.*, 1981, 45, 1327-1324. *Am. J. Enol. Vitic.*, 1980, 31, 28-37).

1410. S. Hara (1981). HY-1. Hybrid NCYC 1408 x 1407. (*Agric. Biol. Chem.*, 1981, 45, 1327-1324. *Am. J. Enol. Vitic.*, 1980, 31, 28-37).

1411. DSM (1982). DSM 70466. Bordeaux strain.

1412. DSM (1982). DSM 70461. Madeira strain.

1413. DSM (1982). DSM 70467. Sautemes strain.

1414. DSM (1982). DSM 70464. Tarragona strain.

1415. DSM (1982). DSM 70468. Tokay strain.

1431. C. Tusting (1982). French cider yeast.

1451. Weston Research Labs (1982).

1499. G. G. Stewart (1983). BB17. From Labatt's brewery.

1516. British brewery (1984).

1529. CBS (1984). CBS 6128. Baker's Yeast.

1530. CBS (1984). CBS 6131. Baker's Yeast.

1533. G. M. Gadd (1984). ED 66.20a.

1534. G. M. Gadd (1984).

1593. ATCC (1986). ATCC 60530.

1765. D. H. Grout (1987) ATCC 96819.

2551. K. Hickson (1994). From 'Teff'.

2589. CBS (1994). *Saccharomyces cf. cerevisiae*. CBS 426. From honey.

2593. M. Rhymes (1994). Flocculent isolate from NCYC 1168.

2645. British brewery (1994).

2657. Yogurt manufacturer (1994).

2740. CECT (1997). CECT 1170, DCL 740.

2743. CECT (1997). CECT 1482, IFI 460.

2776. F. C. Odds (1997). MAS 1.

2777. F. C. Odds (1997). MAS 2.

2778. F. C. Odds (1997). MAS 3.

2779. F. C. Odds (1997). MAS 4.

2780. F. C. Odds (1997). MAS 5.

2798. F. C. Odds (1997). MAS 6.

2799. CBS (1997). CBS 2247, CL 504, CCRC 21961, DBVPG 6172, IFO 1991, NRRL YB-4237, NRRL YB-4254, VKPM Y 47.

2826. CECT (1998). CECT 1483, IFI 649.

2830. CECT (1998). CECT 1683, IFI 270.

2843. UK Food Industry (1998).

2847. F. C. Odds (1999). J980380.

We have also used the FLO1 gene and ADH in the yeasts *Saccharomyces cerevisiae* from strains having fully different characteristics from W204, such as those listed in the application 8, 9, 108 and 109. Those strains have characteristics such as: they auto-distruct themselves, they are diploid, resistent against drugs, they have important characteristics for cell division, permeability to aminoacids and different fermentation times and characteristics.

The most well-known flocculation genes are FLO1 or FLO1S or FLO1L, FLO2, FLO3, FLO4, FLO5, FLO6, FLO7, FLO8, FLO9, FLO10, FLO11, PKC1 and Lg-FLO1. When referring to microorganism, we will also refer to bacteriae, fungi and archae having these and other flocculation genes.

In the present application, we claim the patent not only for microorganisms, cassettes, vectors, plasmids and processes based on genes already mentioned in other patents, such as FLO1, FLO1s, FLO1L and PKC1. In the present application, we also claim a patent for microorganisms, cassettes, vectors, plasmids and processes based on genes which had never been mentioned in patents of this kind (of the nature adding flocculation genes to promoters regulated by the fermentation process), such as FLO2, FLO3, FLO4, FLO5, FLO6, FLO7, FLO8, FLO9, FLO10, FLO11 and Lg-FLO1. These genes have different characteristics from genes FLO1, FLO1s, FLO1L and PKC1. These different characteristics are, among others, the ability to deflocculate only with sugar, with no need of acids. This characteristic is very important to allow the process to be reversible by adding substract from the next fermentation cycle. Therefore, the yeast can be re-used with no need of one more stage in the production cycle which should occur at the time of adding the acid. Furthermore, the lack of need of adding acid makes the process become cheaper and simpler.

In the present application, we also claim a patent for the combination of genes FLO1, FLO1S and FLO1L, as used in Watari's patent, but combined with other promoters. Watari has used such genes with the ADH promoter. In the present application, we use these genes with promoters HSP30p, MOX and pMET3. All these have much different characteristics from ADH, generating unexpected effects. The ADH promoter is restrained by the presence of glucose and therefore is on when there is no glucose in the medium. The use of the ADH promoter to regulate the flocculation gene allows the flocculation to only occur when the level of glucose in the medium falls to very low levels.

The use of the HSP30 promoter together with flocculation genes generates a different effect, since this promoter is controlled by other factors, such as thermal shock, lack of nitrogen and other nutrients, fall of pH and high ethanol concentration. For these characteristics, it allows to control the end of the fermentation

process and the start of flocculation through other mechanisms. It is therefore allowed to end fermentation not only as required when the glucose ends, but before or afterwards.

The use of the MOX or pMET3 promoter together with flocculation genes generates a different effect, since these promoters have stronger expression than ADH, generating larger production of hyphas for flocculation.

In the present application, a patent has also been claimed for the genetically modified microorganism, by using genes sfl1ou, fsu1ou, fsu2ou, tup1ou, cyc8ou, cka2 or FMC1, which had not been the scope of any patent application in the state of the art, combining such genes with regulated promoters for effects characterizing the end of the fermentation process. In the present application, these genes were regulated by a range of promoters, HSP30, pMET3, MOX and ADH.

The union of the coding part of all flocculation genes to promoters which are started or restrained, depending on characteristics of chemical composition of the medium, pH or by physical excitations, bring relevant characteristics for various industrial processes.

Some of the promoters with said characteristics are the following: ADH, Mox or HSP30p. Such promoters can be started or restrained at the end of a fermentation process, by adding flocculation genes to the coding part, causing the flocculation and settling of the microorganism in the bottom of the vessel in which said fermentation process is taking place. Said effect is interesting, among other things, to separate the microorganism from an eventual substance which production is desired.

Such substances might be produced by the process of putting nutrients and microorganisms in a fermenter, in which microorganisms will produce interesting substances while being fed with nutrients.

After nutrient consumption, the promoter activates flocculation genes and the microorganism would settle down in the bottom of the fermenter, separating from the substances of interest. The biological process under which the microorganism consumes nutrients can be ruled by aerobic or anaerobic breathing, and the substance of interest may be the result of an alcoholic fermentation or consequent substances from the transcription of specific genes. E. g., coding genes producing proteins of pharmaceutical, industrial, cosmetic, agricultural interest and others.

In this process, we not only refer to alcoholic (anaerobic) fermentation, but also to aerobic fermentations.

It is important to stress that, in the second case, recombinant DNA skills can be used, as well as for the expression of already existent genes in the microorganism. Production methods are fully different, although both are known by the word fermentation.

In the anaerobic fermentation, alcohol is produced from the

conversion of sugar in a breathing process. In the second process, there is the transcription of a specific gene from the microorganism, and the final product is not the result of a metabolic cycle of cell breathing. Aerobic breathing consists of a fully different metabolic process. That difference allows the production of other substances than alcohol, such as pharmaceutical products (e. g. insuline, antibiotics, growth hormones and others), industrial and agricultural enzymes (such as xanthan gum, aminoacids, organic acids, flavors, vitamins, bioinsecticides and many others). The anaerobic breathing process mentioned by Pereira (2000) is not appropriate to the production of said substances, but only alcohol.

Furthermore, we use other flocculation genes such as FLO10, which has another effect by following other flocculation genes.

The gene FLO10 is deflocculated by the introduction of sugar again into the medium. This effect is important, since for other applications it allows to re-use microorganisms. The FLO1 gene can only be used for beer industry, since yeasts are not re-used there.

The FLO1 gene can deflocculate with the introduction of sulfuric acid, but the FLO10 gene elliminates this costly stage, since it can already deflocculate in the next production stage, when sugar is re-introduced into the medium for the next fermentation.

Another aspect is the use of promoters aside from ADH, such as MOX and HSP30p. These promoters have different characteristics. Mox, although also regulated by the presence of glucose such as ADH, has a more intense expression than ADH, causing higher flocculation rate. The HSP30p promoter has other interesting characteristics, since it can be activated not only by the lack of glucose, but also by the lack of nitrogen, or it can be activated by physical stimulation, such as thermal shock or pH fall.

Another characteristic is the use of not only wild lines, such as claimed by Pereira, but also non-wild lines, with essential consequences to the economical feasibility of the project.

The non-wild lines are most easily manipulated in laboratories, and can therefore be used in recombinant DNA skills, as well as the production in controlled aerobic fermentations for the production of pharmaceuticals, industrial enzymes and other products.

Furthermore, this kind of production of non-wild lines (laboratory), in opposition to the wild lines referred to by Pereira, are ideal for the production in a controlled production environment concerning the risk of contamination of the environment with genetically modified organisms.

The production referred to by Pereira (2000) occurs in large

industrial vats in sugar-alcohol usines where there is no control of biological material leakage to the environment. Much on the contrary, in centrifuges and vats of a sugar-alcohol usine, there are constant leakages and evaporation of biological material. This characteristic can make the use of this type of skill by biosafety control organisms become unviable.

Theoretically, it would be possible to control the leakage to the environment at sugar-alcohol usines, but said control would be economically not feasible.

Laboratory lines do not survive in such vats, but are ideal for controlled laboratory fermenters, in which proteins such as pharmaceuticals and industrial enzymes are produced, since said non-wild yeasts have a known metabolic profile, which is essential for the existence of a rigorous control of production quality of a medicine. In the production of medicine, the control of quality and contaminants is essential to obtain FDA approval. In the production of industrial enzymes, there is also such a control, since many of them have high added value. In this kind of production environment, contamination control is essential and therefore the risk of contamination from the environment to the fermentation vat and from the vat to the environment with a genetically modified microorganism is minimum.

Furthermore, another advantage of the non-wild lines is that they have well-known markers, thus facilitating to select modified cells and avoiding the use of markers with higher public health risks. Another aspect of the wild lines is that they are diploid and not haploid, such as laboratory lines. Being diploid, wild yeasts have higher productiveness rate, but demand much more work to make a genetic modification, since two copies of the gene must be modified. In haploid yeasts, there is only one copy to be modified. Wild yeasts also have a different characteristic in terms of natural selection in fermentation tanks, besides being less stable to a plasmid change.

One of the ways to practice the invention is to put in a plasmid promoters such as ADH, MOX or HSP30p together to the coding part of flocculation genes such as FLO1. FLO1 has insufficient flocculation index in various yeasts, since this gene presents a repeating portion and frequently suffers recombinations. FLO1L or FLO1S gene has much higher efficacy. However, genes FLO1, FLO1L and FLO1S only deflocculate in the presence of an acid, such as sulfuric acid.

The FLO10 gene deflocculates with the presence of sugar, generating deflocculation by the raw material of the next producing cycle itself, making the fermentation process become simpler and cheaper.

Example:

The gene and the promoter are inserted in a plasmid with a

gene marker, which is then introduced into a yeast. Cells are grown in a medium with the specific antibiotic of the marker to only select genetically modified yeasts. Said selected yeasts are then able to conditionally flocculate, following the expression characteristics of the promoter. They can flocculate when glucose ends, when nitrogen ends, when there is a thermal shock or an abrupt fall of pH. Anyway, it is interesting that such flocculation activators occur at the end of the fermentation process, to make best possible use of nutrients. Once flocculation is activated, the microorganism settles down in the bottom and the final fermentation product can be taken out from the vat by the higher part of the fermentation vat.

Example of embodiment step by step

In this recombinant plasmid construction (YEp), in which we associate a promoter regulated by glucose, such as ADH and the FLO5 gene, we use the sites for restriction enzymes NdeI and HindIII at the ends of the promoter and thus the linkage of the promoter is made at position 5' to the flocculation gene at position 3', on the same reading frame. On position 3' of the flocculation gene, a stop codon has been introduced. In that construction, a plasmid with supplementation mark for Uracil auxotrophy (URAA3) and antibiotic resistance gene is used. These two marks allow the complementation of laboratory yeasts with auxotrophy for uridine and uracil and also the selection of transformed strains by the resistance mark against inhibitting agents.

Example of laboratory test

The flocculation cassette has been introduced into the yeast by means of electroporation, using the following standards: 1.5 kV, 200 ohm and 1.2 cm tub. The transformed yeast has been selected for the lack of uridine and uracil in the culture medium. The fermentation process has taken place in a minimum medium supplemented with glucose. After 17 hours of culture, flocculation occurred. The use of the FLO10 gene makes the introduction of glucose after the occurrence of flocculation, making the flocculation process to be visibly reversible. The transformed strain has remained stable during culture.